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APCBEE Procedia 5 (2013) 491 – 496

Procedia APCBEE

www.elsevier.com/locate/procedia

ICESD 2013: January 19-20, Dubai, UAE

Alterations in Antioxidative Defense System of *Anabaena Variabilis* in the Presence of Heavy Metals

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Abstract

A general increase in the level of heavy metals posses a pervasive threat to the natural ecosystem, although many heavy metals, when in trace amount are essential for various metabolic process in organism, they create physiological stress leading to generation of free radical, when in high concentration, stress in turn induces the production of reactive oxygen species. Cyanobacteria posses an effective stress combat system to cope with pressure by the help of cascade of antioxidants where superoxide dismutase act initially followed by catalase and peroxidases. The present study was conducted to evaluate the effects of heavy metal ions on the levels of antioxidant enzymes of the organism. Various effects of alkaline earth metal on cells were depicted by means of variations observed in cell size, shape and their density. The differential response of activities of all studied enzymatic antioxidants was investigated and it was observed that SOD activity showed was less in control and cobalt with passing time whereas in the presence of Mg^{2+},Mn^{2+},Cd^{2+},Hg^{2+},Zn^{2+} the activity was enhanced. CAT activity showed opposite pattern as CAT activity was significantly less in the presence of these metal ions as compared to SOD values, whereas SOD is correlated to the POD activity and the organism grown in Mn^{2+}, Zn^{2+},Hg^{2+} showed similar pattern . This may help in developing a strategy to improve cyanobacterial tolerance towards these metals in order to develop strains so to be used to reclaim the environmental stress.

Key words: Antioxidant; cyanobacteria; heterocystous; free radical; heavy metals.

1. Introduction

There is accumulation of enormous number of contaminants in our environment due to rapid urbanization of our world. Heavy metals hold an exceptional position in that list and are responsible of contaminating soil, water and all food stuffs taken up by the humans [1].

Higher levels of heavy metal can lead to an increased production of reactive oxygen species (ROS) from excited photosynthetic pigments [2]. These ROS can cause oxidative damage to polyunsaturated fatty acids in the cellular membrane and breaks in DNA strands[3].

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doi:10.1016/j.apcbee.2013.05.083

The present study included tests of the effects of the heavy metal stress on activities of SOD, CAT and POD. The objective of this study was to explore the survival strategy of test organism, *Nostoc muscorum* under seven heavy metal chlorides (Zn, Mg, Co, Mn, Hg, Pb & Cd) stress by alterations in the activities of superoxide (SOD, EC 1.15.1.1),catalase (CAT, EC 1.11.1.6) and peroxidases (POD, EC 1.11.1.7). Some significant morphological changes was observed according to the degree of toxicity degree of toxicity caused by the test metals. Cyanobacteria exhibit an extraordinary resistance to many environmental factors. A better understanding of the biological effects and response mechanisms of cyanobacteria to heavy metal exposure could be used to develop these bacteria for use in bioremediation.

2. Materials and methods

2.1. Test organisms and growth conditions

The culture of heterocystous cyanobacterium, *Anabaena variabilis* isolated and purified from the paddy field of Barabanki, uttar pradesh India. Cultures were grown in nitrogen-free BG_{11} medium [4] under heavy metal stress, namely, Zn, Mn, Mg, Hg, Co, Cd & Pb as chlorides (4.0µM concentration) no stress culture served as control. Cultures were maintained at 27°C±1°C under fluorescent illumination of 30-40 $\mu Em^{-2} s^{-1}$ provided by fluorescent tubes exposed to a 14 h light /10 h dark photoperiod and swirled manually for five minutes, thrice daily. The cultures were raised in bulk and the 11-day-old cells (exponential phase) were harvested, and centrifuged. This was used as inoculum for all the experiments; the results are presented as means of triplicates.

2.2. Microscopic studies

To observe the structural changes in the test alga in the presence of different stresses, a thin film of algal culture in exponential phase was prepared on slide by immersing the cells in cedar wood oil commonly, a high resolving microscope of 40X resolving power is used to view the organism.

2.3. Enzyme extraction

Fresh biomass was harvested after 11 days (logarithmic phase) of incubation by centrifugation at 10,000 rpm for 5 minutes and the pellet was suspended in potassium phosphate buffer (50mM, 7.8 PH). The homogenized samples were centrifuged at 15,000 Xg for 30 min at 4^{o}C, and the resulting supernatant containing antioxidant enzymes was used for further assays.

2.4. Estimation of SOD activity

SOD activity was assayed by the method of Giannopolitis and Ries (1977) [5]. Absorbance was read at560 nm.

2.5. Estimation of CAT activity

Catalase activity was estimated by measuring the consumption of H_2O_2 (extinction coefficient 39.4 mM-1 cm-1) at 240 nm for 3 min[6].

2.6. Estimation of POD activity

Peroxides activity in a reaction mixture(3ml) containing 16mM H_2O_2,10Mm Pyrogallol and crude extract was determine spectrophotometrically [7] increased activity was measured at 430nm.

2.7. Estimation of APX activity

The activity of ascorbate peroxidase was assayed Chen and Asada (1989) [8]. Decrease in absorbance was

measured at 290 nm.

3. Results

Mercury being the most toxic of all the test metals, caused maximum reduction in growth of the test microorganism, as was apparent by yellowing and fragmentation of filaments. On comparing with control (Fig.1a), cultures containing Cd, Pb, and Hg stress showed distorted shape cells (Fig. 1 b, f, g) indicating large production of ROS, cobalt proved to be more toxic as shrinkage of cells can be seen in both vegetative as well as heterocystous cells (Fig. c) and showed somewhat less dense cells than control (Fig.1a). The results related to the effect of magnesium, manganese and zinc on test culture is depicted in Figures 2 d, e, h. These metals overcame the levels of toxicity may be due to presence of methionine which may result in binding of the metals with sulphydryl groups. Further, the polysaccharides may act as chelators [9].

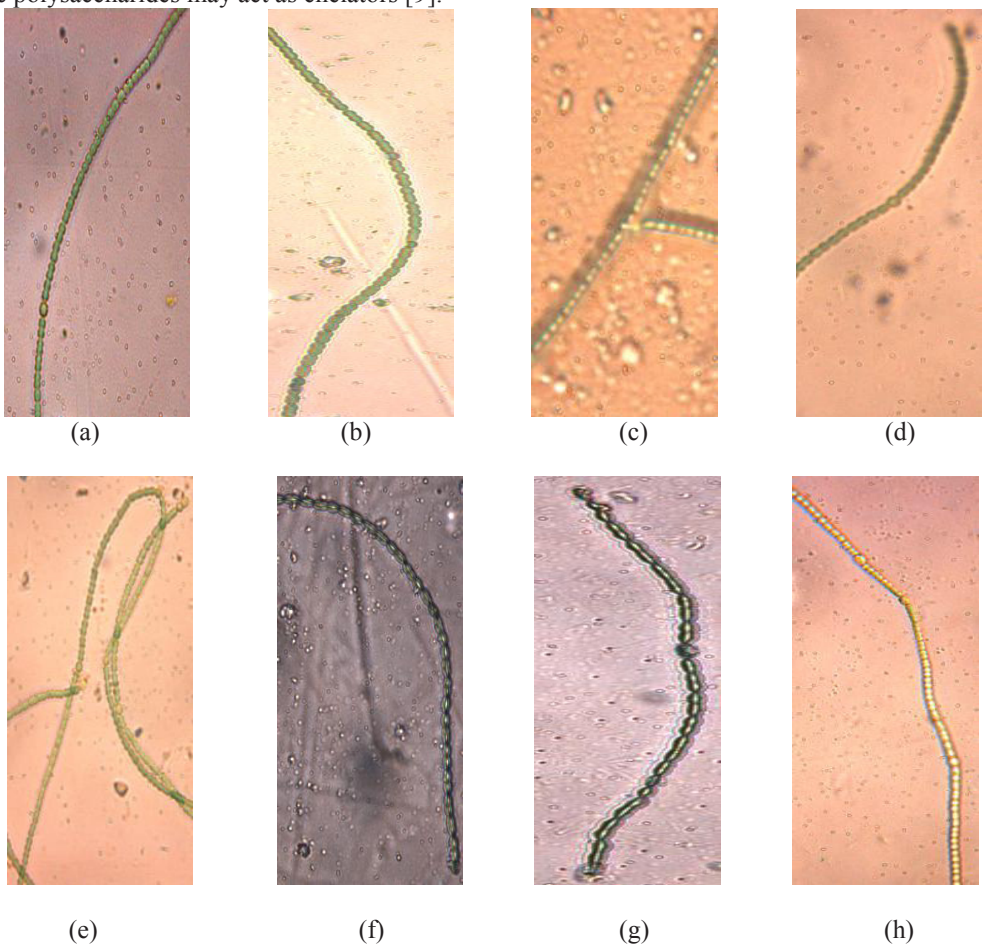

Fig. 1. Morphological changes in A*nabaena variabilis* under chlorides of heavy metal stress (a) Control, (b) Cd, (c) Co, (d) Mg, (e) Mn, (f) Pb, (g)Hg and (h) Zn.

Figures 2, 3, 4, and 5 compiles data on the impact of different heavy metal stress on antioxidant enzymatic activities (SOD, CAT, POD, APX). Increased SOD activity was observed in Mg, Mn, Cd, Zn, and Hg stressed cells in comparison with control. Lead stressed cells initially increase, then decreased in the activity,. Among all the experimental metals *A. variabilis* showed maximum SOD activity in the presence of mercury. Magnesium, cobalt and zinc seemed to stimulate scavenging activity in the organism, more than control. SOD activity was significantly related with peroxides and negatively with CAT. In contrast to SOD activity CAT activity showed decrease with the passage of time. Control and cobalt irrespective of other metals behaviour showed gradual increase in the CAT

activity. In both the enzymatic mechanism, mercury showed the highest level of toxicity, among all the seven metals tested in the test organism. The POD activity registered an increase in magnesium and cadmium containing cultures respectively as compared to control. Zinc and mercury initially showed increase in the scavenging activity which later declined. However, this nature is contradictory to the SOD activity pattern. The result depicted from Fig. 6 showed a regular decrease in APX activity in control initially than enhancement later on. However, some metals containing test alga showed supportive behaviour in scavenging activity as it can be predicted from the graphs of manganese and cobalt. Mercury showed strong toxicity by sharp decline in APX activity. Cobalt showed drastic inclination in activity which reflects its great adaptation in the toxic environment. Followed by mercury, cadmium and zinc showed almost similar levels of toxicity.

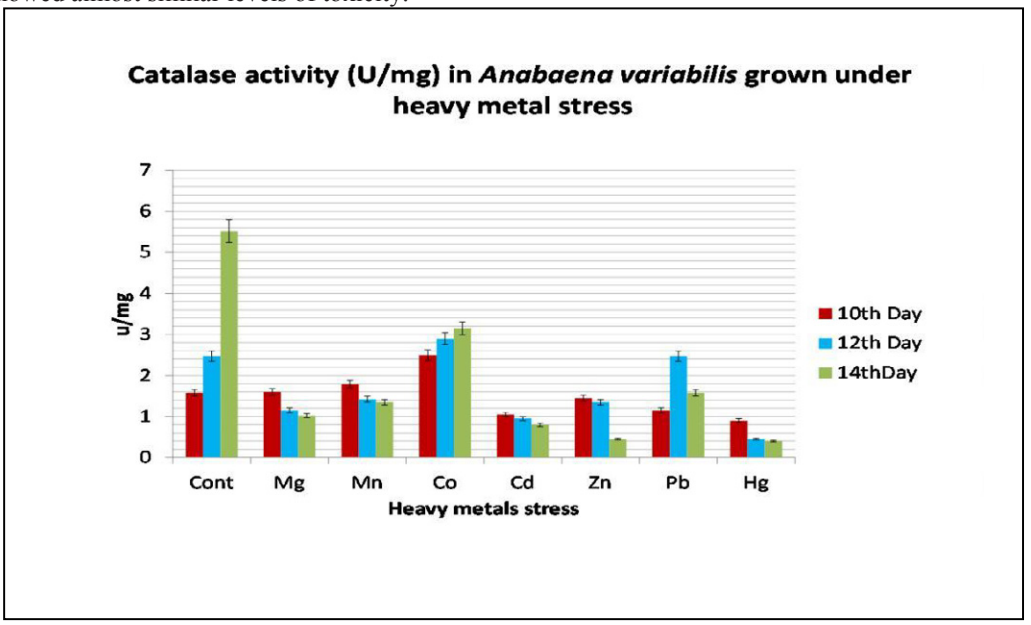

Fig. 2.

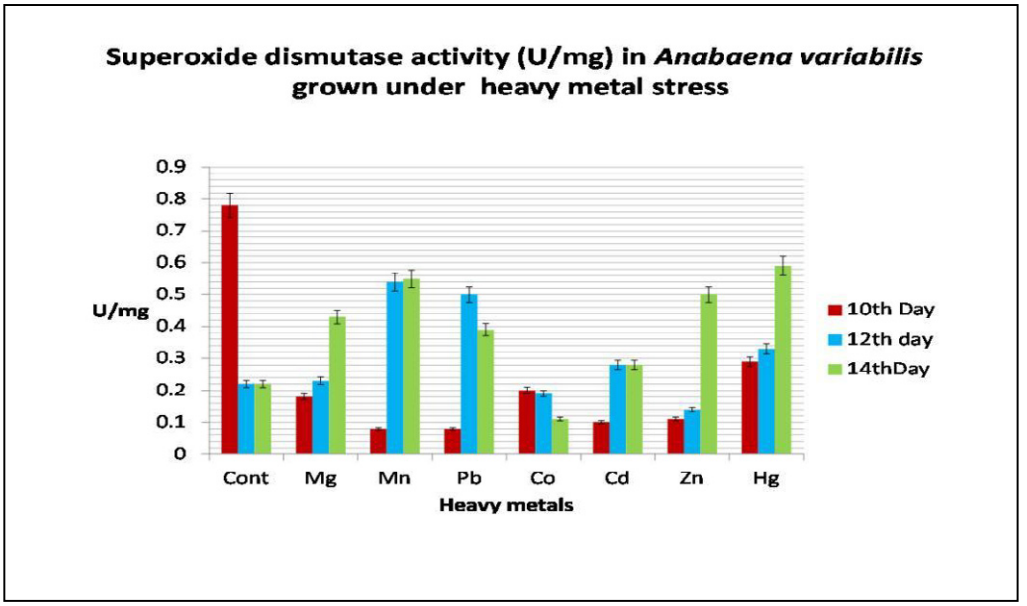

Fig. 3.

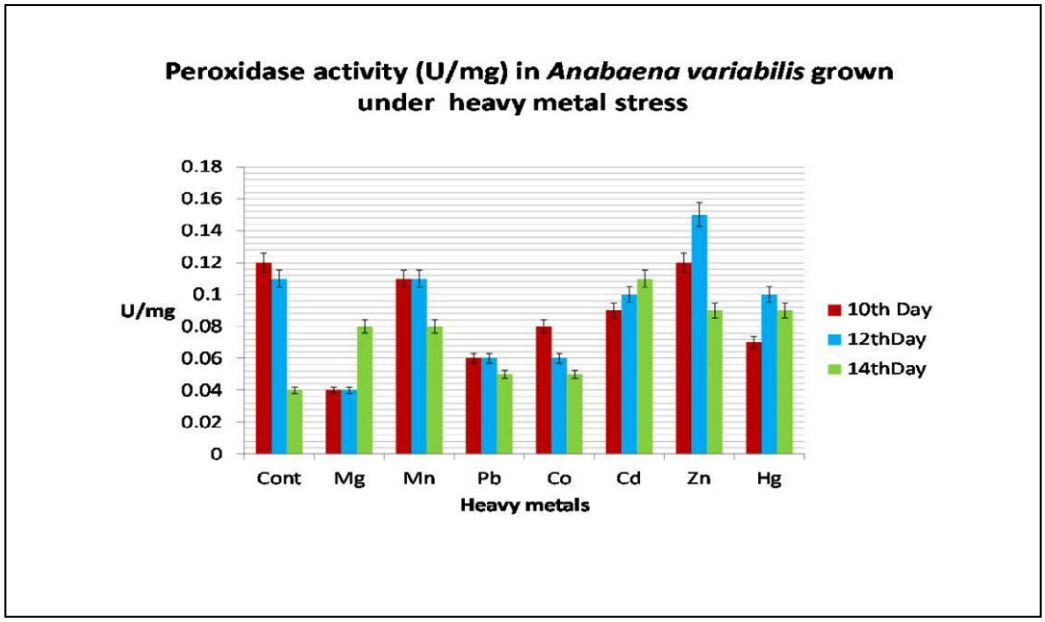

Fig. 4.

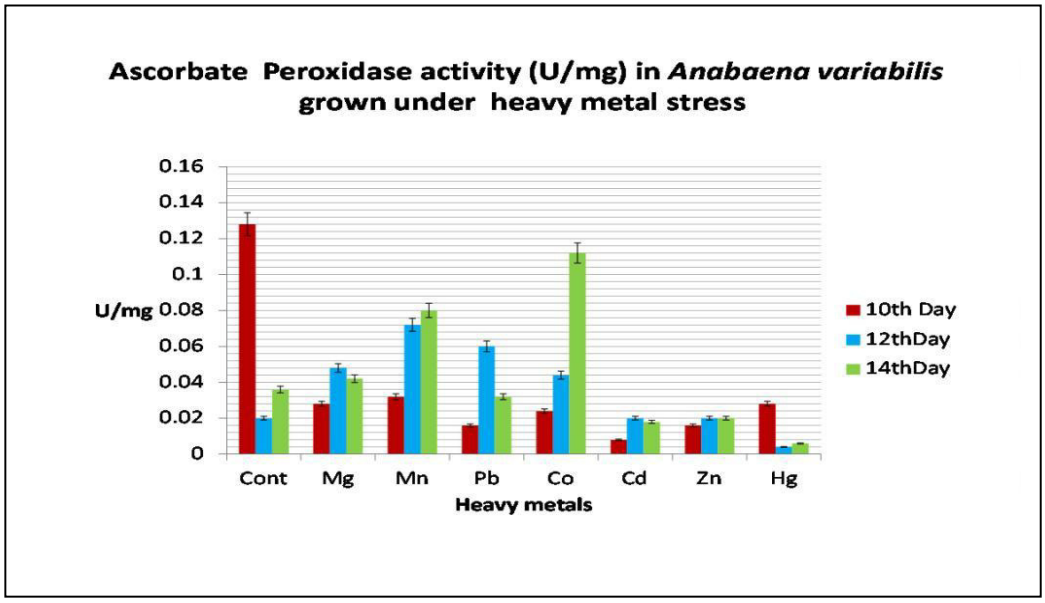

Fig. 5.

4. Discussion

These results offer support to the contention that growth reduction is responsible determinant for the metal toxicity. The degree of response is deep dependent on the amount of metal that traverse for reduction in growth in cyanobacteria after exposure to heavy metals. The toxicity of several heavy metals has been reported to reduce to phytoplankton with the help of chelators [10], The increase in SOD activity after metal treatment could be due to production of O_2 anion whose detoxification is necessary for the growth of organism [11], In higher plants, heavy metals induce generation [12] thus it converted into peroxide by the activity of SOD [13], and further into hydroxyl

radical and singlet oxygen by POD and CAT .

These result offer support to the contention that growth reduction is responsible determinant for the metal toxicity. This reduction could be due to binding of test metal to sulphydryl group which is responsible for cell division in plants including cyanobacteria [14]. This shows that adaptation of the cell to metal stress is very much depending on the auto oxidation defense mechanism. Cyanobacterial cells could be utilized commercially to prepare UV- protective creams because of its large antioxidant potential as shown in the present study. Herbs having polyphenolic constituents are antioxidant in nature and are used for combating the deleterious effects of ultraviolet radiations thus producing photoprotective effects and creams have been developed [15].

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